STARCH FORMATION IN ZYGNEMA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 238

HELEN BOURQUIN

(WITH PLATE XXVII)

Between 1880 and 1895 much literature on the subject of starch formation appeared. At that time certain problems in regard to the origin of starch grains in algae arose which have not yet been settled. The present investigation of *Zygnema* was undertaken because its chromatophore appears to be typical of many algae, and it is so large that the possibility of error in cytological work should be minimized.

History

The most important investigations on starch formation were made by MEYER (3, 4). One of his conclusions is that starch is always formed by the plastid by secretion. Although he confined most of his attention to angiosperms, he believed this to be equally true for algae (4). He concludes that the pyrenoid is reserve protein material which is formed by the plastid and is used by the cell after its supply of starch has been exhausted. In his opinion it is homologous with the protein crystals formed in the plastids of many of the lower monocotyledons, which are likewise utilized after all the starch grains have disappeared. The plastid itself he sees as a colorless or slightly yellowish honeycombed structure ("vakuolig-poröse"), which contains chlorophyll in the form of granules. Other investigators agree that the pyrenoid is implicated in some way in the formation of starch in algae.

SCHMITZ (7) finds a specific pyrenoid substance which is laid down locally in the substance of the plastid in such quantities that the mass appears as a structure more or less sharply differentiated from the plastid. Although formed by the plastid, this pyrenoid substance is living and takes an active part in starch formation. He adds that in many cases the plastid forms starch without the intervention of the pyrenoid, and that pyrenoids often appear in

plastids in which no starch is formed. He thinks that the pyrenoid is homologous with the protein aleurone crystals of the lower monocotyledons, and he places the pyrenoid substance in the same chemical group as chromatin.

SCHIMPER (5, 6) describes the pyrenoid as a crystalline substance which rises *de novo* in the cytoplasm, and which in turn gives rise to the sheath of starch grains surrounding it. The plastids of algae always form it in the process of starch formation. If abundant, it crystallizes; if thin, it passes into starch without crystallizing and its presence cannot be demonstrated by the microscope, as is possible when it is in crystalline form. It and its surrounding sheath of starch grains are structures peculiar to algae and not homologous with any structures found elsewhere in plants. He believes that it does not belong to the same group as chromatin.

According to TIMBERLAKE (8) there is no differentiated chromatophore in *Hydrodictyon utriculatum*, but the chlorophyll is distributed through the whole peripheral protoplasmic layer of the cell (p. 623). Just before zoospore formation the pyrenoids disappear. They rise *de novo* when the zoospores germinate. The substance of the pyrenoid is changed into starch grains by "processes not understood." He is inclined to believe that the pyrenoid is an active independent cell organ whose function is to produce starch.

YAMANOUCHI (9) finds a plastid in *Hydrodictyon africanum*, a plant like *H. utriculatum* in all essential features. In its early stages the plastid is denser in the outer regions than in the center and is irregular platelike or spindle-like in form. It may produce either reserve starch grains or pyrenoids. The two are not recorded in the same plastid.

CHMILEWSKIJ (1) believed that the starch grains in *Zygnema* are formed wholly from the substance of the pyrenoid, plates of which extend between the starch grains, giving a starlike structure.

Miss MERRIMAN (2) figures the starch grains as entirely surrounded by cytoplasm except on the side abutting on the pyrenoid. Since this work of Miss MERRIMAN'S has been accepted as the standard on nuclear division in *Zygnema*, it seems necessary to emphasize the fact that she makes no claim of having interpreted

the chromatophore correctly. She has figured it incidentally as it appears to the casual observer.

Material and methods

My observations were made on several species of *Zygnema*. The material came from the vicinity of Chicago and from Dr. TRANSEAU'S laboratory. It was killed in 1 per cent chromoacetic acid, part of it at 11:00 P.M. to secure cells which were dividing, and part of it at 11:00 A.M. to catch the cells in an active vegetative condition.

Filaments were mounted whole in Venetian turpentine and stained with anilin blue and Magdala red and with gentian-violet and anilin blue in order to differentiate the parts of the chromatophore. Iron hematoxylin was also used for some preparations. Most of my observations were made on the whole filaments. Many of the drawings are optical sections of whole chromatophores. The advantage in this method lies in the fact that the true sizes and relations of the starch grains and the exact extent of the pyrenoid in the chromatophore can be ascertained by focusing up and down. The use of sectioned material exclusively might lead to errors of interpretation by taking the transverse section of a grain for a small one, or by failure to see the full extent of the pyrenoids. Longitudinal and cross-sections of cells 3 μ thick were also made and were stained with gentian-violet and safranin, iron hematoxylin, and Flemming's triple stain. An 8× ocular and a 2 mm. objective were used for examining the preparations.

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Description

The chromatophores lie, one on each side of the nucleus, in the middle of the cell, suspended there by means of bands of cytoplasm which radiate from them to the layer of the cytoplasm along the cell wall (fig. 1). Under low-power lenses these bands appear to be part of the chromatophore and have led writers of textbooks to speak of the star-shaped chromatophore of *Zygnema*. The chromatophore is really round or oval in shape. It is a plastid containing imbedded in its substance a pyrenoid, which lies near

the center, and starch grains which radiate out from the pyrenoid (fig. 7).

Staining differentiates the plastid from the pyrenoid and the starch grains. The plastid is stained a bright blue by anilin blue, gray by iron hematoxylin, and faintly violet by gentian-violet when the starch grains are stained very deeply violet. It is colorless when the starch grains are only faintly violet. The latter are stained a yellowish red by Magdala red and violet by gentian-violet. The pyrenoid stains red with Magdala red and safranin and dark blue with iron hematoxylin. The plastid is differentiated from the cytoplasm which surrounds it by its structure, the plastid appearing homogeneous (figs. 1, 7).

It is most easily seen in chromatophores which are dividing (figs. 17, 18), but any chromatophore in which the starch grains are not closely packed shows it. A layer of the substance of the plastid always surrounds each starch grain and the pyrenoid, thus separating the starch grains from each other and from the pyrenoid. This layer can be demonstrated even when the starch grains are most closely packed (figs. 1, 2, 3, 10).

The pyrenoid is a homogeneous structure which stains with different intensity in different parts of its mass, so that one part of it will be dark blue and another gray, or one part lighter red than another. It usually forms a compact, irregularly oval or round mass in the center of the plastid from which it is sharply differentiated. An examination of the figures will show that it may vary greatly from these shapes, but it does not extend up between the starch grains as CHMILEWSKIJ (1) believed. Staining the chromatophore with Magdala red, which stains the pyrenoid, and with anilin blue, which stains the plastid, proves this conclusively. Occasionally, when there is a large space between two starch grains, tongues of the pyrenoid extend a short way up between them (fig. 8). These tongues never reach the periphery of the plastid, come into direct contact with a starch grain, or surround one.

I have not found more than one pyrenoid in a plastid unless that plastid was about to divide, division of the pyrenoid always immediately preceding the division of the plastid (figs. 17, 18).

In the majority of chromatophores the starch grains lie radially about the pyrenoid, with their broad end toward the periphery of the plastid and the narrow end abutting on the pyrenoid (figs. 1, 2, 10). They are approximately equal in length, but vary in shape from cuneate to trapezoidal to rectangular (grains *l*, figs. 2, 3, 10, 16). Their opposing faces are straight; the outer faces are rounded or straight.

In many plastids there are minute grains in the periphery of the plastid (figs. 8, 10, 15, 16). Grains of all sizes from minute grains to the larger grains are also found. These intermediate grains are all cuneate in shape, if they lie between the larger grains, and always occur near the periphery of the plastid (figs. 8, 15, 16). Occasionally the starch grains are clustered irregularly about the pyrenoid (figs. 8, 15). In this case also the smaller grains are found in the periphery of the plastid.

Discussion

There are two opinions about the origin of starch grains in algae. The majority of investigators believe that the pyrenoid is concerned in starch formation. MEYER believes that the pyrenoid is not concerned in starch formation. The pyrenoid may be implicated in starch formation in either of two ways. Its substance may be changed into starch, that is, it may form starch by fragmentation, as SCHIMPER (5, 6) and TIMBERLAKE (8) believed, or it may be an active starch-former by secretion.

In *Zygnema* the substance of the pyrenoid is not changed into starch. This is shown by a comparison of the shape and position of the starch grains and the shape and position of the pyrenoid and by an examination of the pyrenoid itself. If the starch is formed by fragmentation of the pyrenoid, there should be such a similarity in the contour of the two that the starch grain could be fitted into the edge of the pyrenoid as the parts of a puzzle fit together. If the grains are chipped out so that they come to lie radially about the pyrenoid, bands of the pyrenoid should lie between the starch grains. Even though the pyrenoid were to grow and change shape after the formation of each starch grain,

some newly formed grains would always be found which would indicate their origin in that way.

Typically the pyrenoid forms a round mass in the center of the plastid (fig. 1). The most extreme variations in its shape are figured (figs. 2, 8, 12, 14). In no case is there a striking similarity between the contour of the pyrenoid and the starch grains, nor does the pyrenoid extend between the starch grains in such a way as to suggest that the starch grains have been cut out of the substance of the pyrenoid. Fig. 8 is the most suggestive found.

Although the pyrenoid is stained with different intensity in different parts, the whole pyrenoid stains the same color with any given stain. The narrow band of the substance of the pyrenoid connecting the halves of a dividing pyrenoid never stains deeply (fig. 18). In the vegetative plastid these light and dark areas bear no definite relation to each other or to the surrounding starch grains (figs. 2, 3, 8, 9, 11, 15). Moreover, these light and dark areas are uniformly homogeneous in structure. I believe, therefore, that they do not indicate any change in the substance of the pyrenoid; they are simply regions of different density.

Grains lying radially about the pyrenoid and varying in shape from cuneate to rectangular might be formed by the pyrenoid by secretion were the pyrenoid to form them in the periphery of the plastid and add to them centripetally, or to form them at the center of the plastid and add to them continually on the inner edge, such additions pushing them automatically toward the periphery of the plastid. The first manner of formation is impossible since the pyrenoid is always confined to the center of the plastid and in no case was seen to approach the periphery where the smaller grains of starch occur. The nearest approach to such a situation is seen in figs. 8, 14. The second possibility cannot exist because the small grains are never found next to, or near, the pyrenoid. They were found without exception in the periphery of the plastid. Since there is no indication that starch grains ever split, they could not have been derived indirectly from the pyrenoid in that way. The fact that the plastid separates the pyrenoid and the starch grains becomes significant when added to these proofs that the pyrenoid does not take part in starch formation in *Zygnema*.

Everything bears out the following theory concerning the origin and growth of the starch grains in the form. The plastid gives rise to minute starch grains in its periphery, either between the larger grains (figs. 15, 16) or entirely beyond them (figs. 8, 11). Their growth seems to be regulated by their position in relation to the larger grains and to be a mechanical matter. Figs. 5, 6, 13 show how the shapes of the grains seem to fit naturally into the place occupied. If the small grains lie between larger grains, the plastid adds to them in such a manner that they become cuneate in shape. The enlargement on the inner face continues to be more rapid lengthwise of the grain than laterally as it grows down between the grains toward the center of the plastid, so that when it attains the length of the large grains it is still cuneate. A comparison of grains *t* in figs. 3, 8, 14, 16 shows a complete gradation in the lengths of the grains from the shortest to the longest, so that a perfect series could be arranged were the grains removed from their respective plastids.

After lengthening the grain in the manner described, the plastid begins to add most rapidly to the narrow base laterally, so that the grain becomes trapezoidal and then rectangular in shape. In rare instances this basal broadening may continue until the grain is once more trapezoidal (fig. 2). Grains *l* in figs. 2, 10, 16 show every stage in the change of shape just mentioned. If the small grains are formed above larger grains, they grow rectangular or oval, pushing the grain below toward the pyrenoid. This is the unusual rather than the normal method of growth, however.

Summary

The chromatophore of *Zygnema* is a plastid containing imbedded in its substance a pyrenoid which lies near the middle, and starch grains which usually lie radially about the pyrenoid.

The pyrenoid cannot take part in starch formation because it is always confined to the center of the plastid and is separated from the starch by the plastid, and because the small young grains of starch are always found in the periphery of the plastid. The plastid therefore must form these minute starch grains.

The starch grains come to lie radially about the pyrenoid in the following manner. The plastid adds to them in such a way that they become cuneate in shape. In this manner they grow down between the starch grains already formed until they are of the same length as the large grains. The plastid then broadens them at the base until they become rectangular in shape.

AGNES SCOTT COLLEGE
DECATUR, GA.

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EXPLANATION OF PLATE XXVII

The drawings were made by the aid of the camera lucida, the magnification being ×1040. The abbreviations used are as follows: *c*, cytoplasm; *r*, pyrenoid; *n*, nucleus; *p*, plastid; *g*, starch grain; *t*, starch grain growing in length toward center of plastid; *l*, starch grain growing broad at base; *s*, minute starch grain.

FIG. 1.—Optical section of cell showing relation of cytoplasm, nucleus, and chromatophores, and showing typical chromatophores packed with large starch grains radiating from pyrenoid.

FIG. 2.—Optical section of chromatophore showing grains of varying width at base.

FIG. 3.—Optical section of chromatophore showing grains of varying lengths and grains which vary in width of base as compared with width of outer edge.

FIG. 4.—Cross-section of chromatophore showing grains of starch in cross-section.

FIG. 5.—Same as fig. 4.

FIG. 6.—Same as fig. 4.

FIG. 7.—Optical section of chromatophore showing several smaller starch grains in periphery of plastid.

FIG. 8.—Optical section of chromatophore showing minute starch grains in periphery of plastid, a pyrenoid of unusual extent and shape, and many starch grains which vary greatly in shape and length.

FIG. 9.—Median longitudinal section of chromatophore showing several small starch grains.

FIG. 10.—Same as fig. 2.

FIG. 11.—Cross-section of cell showing chromatophore in cross-section.

FIG. 12.—Same as fig. 9.

FIG. 13.—Same as fig. 9.

FIG. 14.—Optical section of chromatophore showing another pyrenoid of unusual shape, and starch grains of several different lengths.

FIG. 15.—Optical section of chromatophore showing many small starch grains near periphery of plastid, and large grains which differ in shape because of width of bases.

FIG. 16.—Same as fig. 15.

FIG. 17.—Optical section of plastid in process of division, showing pyrenoid after it has divided.

FIG. 18.—Optical section of plastid in process of division before pyrenoid has completed division.